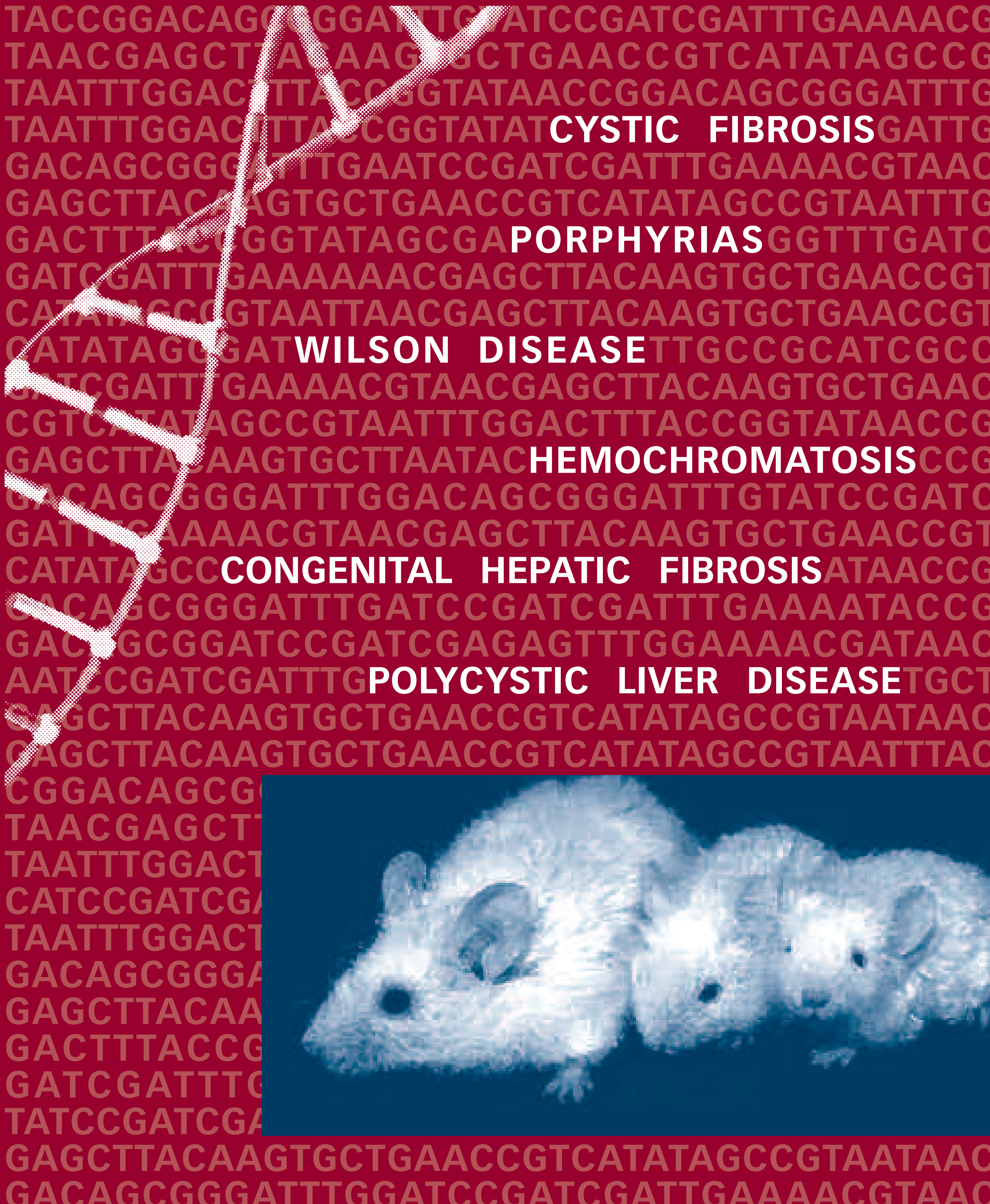

Liver diseases resulting from genetic mutations include hereditary hemochromatosis, Wilson disease, several forms of porphyria, cystic fibrosis, polycystic liver disease, and congenital hepatic fibrosis. The ability to manipulate the genome of model organisms such as mice (shown) allows scientists to add, remove, or modify specific genes that are thought to play a role in these diseases and observe the effects. These studies have revealed the incredible genetic complexity of these diseases, such as the high degree of interplay among various genes and gene products or the contributions of multiple mutations in the same gene, and have thereby identified important causative and modifier genes for these diseases. Design: Joe Vuthipong and Sharon Pope.

CHAPTER 11: GENETIC LIVER DISEASE

INTRODUCTION AND BACKGROUND

Genetic liver diseases include hereditary hemochromatosis, Wilson disease, the porphyrias, cystic fibrosis, polycystic liver disease, alpha-1-antitrypsin deficiency, hereditary tyrosinemia, Alagille syndrome, and several neonatal cholestatic syndromes and inherited diseases of metabolism. Many of these diseases present in childhood and are discussed in other chapters of this Action Plan. This chapter focuses on several genetic liver diseases that present in adolescence or adulthood—hemochromatosis, Wilson disease, the porphyrias, cystic fibrosis, polycystic liver disease, and congenital hepatic fibrosis.

Hemochromatosis is probably the most common inherited disorder among Caucasians, affecting at least 1 in 200 individuals. This disease of iron metabolism is marked by excessive iron absorption and eventual accumulation of toxic levels in the liver, heart, pancreas, joints, and other organs. High iron levels in the tissues are generally not reached until the 4th or 5th decade of life, at which point signs of cirrhosis, heart disease, endocrine failure, or liver cancer may arise. Once the diagnosis is made, hemochromatosis is treated with therapeutic phlebotomy until total body iron stores are reduced to normal. The availability of this safe and effective therapy for a disease that has the potential to cause irreversible liver injury and death from cirrhosis or liver cancer argues strongly in support of early diagnosis.

Wilson disease affects as many as 1 in 30,000 Americans and is caused by loss-of-function mutations in a gene encoding a transport protein that controls hepatic copper metabolism and biliary excretion. Analogous to hemochromatosis, patients with Wilson disease slowly accumulate high levels of copper in tissues throughout the body, typically resulting in clinical presentation in late childhood or early adulthood, and, if untreated, eventually causing irreversible damage to liver, brain, and other tissues. Wilson disease can be treated using oral copper chelators such as penicillamine or trientine and/or oral zinc supplements. In some individuals with fulminant liver failure due to Wilson disease and in those with severe hepatic insufficiency due to the disease, liver transplantation may be necessary and life-saving. For these reasons, early diagnosis is critical in Wilson disease, as it can lead to prophylactic therapy that prevents the disease manifestations.

The porphyrias are a group of inherited diseases caused by defects in the synthesis and metabolism of heme, an iron-binding complex that plays a central role in reactions involving oxygen throughout the body. There are five main forms of porphyria that can affect the liver: acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP), porphyria cutanea tarda (PCT), and erythropoietic protoporphyria (EPP). These are rare diseases, but they have dramatic and disabling clinical features, and they can lead to, or be associated with, end-stage liver disease. Clinical manifestations are due to the high levels of heme precursors (e.g., 5-delta-aminolevulinate, porphyrins) in blood or tissues that can cause acute neurological symptoms (as in AIP, HCP, or VP), skin disease and photosensitivity (as in PCT and EPP), and chronic liver disease. Treatments for the porphyrias range from simple, highly effective therapies that reverse the disease manifestations (e.g., therapeutic phlebotomy for PCT) to more difficult and only partially effective means of ameliorating symptoms (e.g., infusions of glucose and hematin, an oxidized form of heme, for AIP, HCP, VP, and EPP). Some forms of porphyria respond to liver transplantation. Hepatocellular carcinoma risk is increased in most forms of porphyria, especially PCT.

Cystic fibrosis (CF) is an inherited disease that affects 1 in 3,900 newborns and typically leads to severe and progressive pulmonary and pancreatic disease. Until very recently, most persons with CF died in childhood, but with improvements in management of the lung and pancreatic disease, survival into young or mid-adulthood is not uncommon. CF also affects the liver, and, in some cases, liver disease is the major manifestation. CF accounts for 1 to 2 percent of all liver transplants done in children, and liver disease is now the second most common cause of death in persons with CF. The cause of the liver disease appears to be focal inspissation (thickening) of bile and obstruction of biliary flow in the face of steatohepatitis. There are no known therapies for CF-related liver disease.

Polycystic liver disease is an inherited disease marked by multiple scattered cysts of biliary origin in the liver. Polycystic liver disease can occur in association with autosomal dominant polycystic kidney disease (AD-PKD), but most commonly occurs without renal involvement as autosomal dominant polycystic liver disease (AD-PLD). Many persons with polycystic liver disease are without symptoms, but the cysts can become large, causing symptoms of discomfort and liver impairment. Rare cases require liver transplantation. The major gene that causes AD-PLD has been identified recently and is distinct from the genes that cause PKD. The cause of cyst enlargement that leads to clinical complications in some patients is unknown.

Congenital hepatic fibrosis is a rare inherited disorder that occurs together with autosomal recessive polycystic kidney disease (AR-PKD), affecting approximately 1 in 20,000 persons. In most patients, the kidney disease leads to kidney failure and is the major manifestation. In some patients, however, the liver disease is prominent, marked by the presence of extensive fibrosis (without frank cirrhosis) that leads to portal hypertension and complications such as variceal hemorrhage and hepatopulmonary syndrome. The manifestations of congenital hepatic fibrosis are highly variable, and pathogenesis and optimal approach to management are unknown.

Thus, the adolescent- and adult-onset genetic liver diseases are a diverse group of conditions that are uncommon or rare, but cause considerable burden and mortality to those affected.

RECENT RESEARCH ADVANCES

In the last 10 to 15 years, there have been substantial breakthroughs in the understanding of genetic liver diseases. The genes responsible for these diseases have been identified, cloned, and sequenced, and the major mutations have been characterized. Importantly, the identification of these genes has also led to new insights into normal cell biology, frequently advancing knowledge about normal metabolism of iron, copper, and porphyrins, as well as other acquired or metabolic diseases. At present, however, most of these gains in understanding have not led to significant improvements in diagnosis, treatment, or prevention, and they require additional research efforts to close this translational gap.

Hemochromatosis: Classical hereditary hemochromatosis was shown in 1996 to be due to mutations in the *HFE* gene, which codes for a cell surface protein belonging to the HLA gene family. Over 85 percent of Caucasian patients with typical hemochromatosis are homozygous for a single mutation in *HFE* (C282Y), and a lesser proportion are compound heterozygotes for this and another *HFE* mutation (H63D). Mice with deletions in the *Hfe* gene develop iron overload and a clinical phenotype similar to hemochromatosis. Further research has defined the tissue distribution of the *HFE* protein and documented its role in iron metabolism and cell signaling. Importantly, other genes coding for proteins involved in iron metabolism (e.g., divalent metal transporter 1, transferrin receptor-2, hephaestin, hemojuvelin, and ferroportin-1) were found to be involved in hemochromatosis, and rarer forms of this disease have been linked to mutations in some of these genes. Recently, hepcidin—a small peptide secreted by hepatocytes—has been identified as a key sensor of tissue iron status and a modulator of iron absorption in the gut. Normally, hepcidin signals intestinal enterocytes to decrease levels of the proteins involved in iron absorption and macrophage iron release. Elucidation of the factors that regulate hepcidin will likely explain many features of body iron regulation.

A major clinical advance made possible by the discovery of *HFE* was the development of DNA tests for the C282Y and H63D mutations, which are now commercially available. Population-based surveys of serum iron levels and *HFE* mutations are now under way. These surveys will demonstrate the frequency of genetic disease in the general population and determine whether screening is appropriate to identify cases early, thereby enabling the prevention of liver, endocrine, and heart disease due to hemochromatosis. They will also define the degree of penetrance of this gene mutation, i.e., what proportion of persons develops iron overload by a certain age.

Wilson Disease: Wilson disease has been linked to a copper transport protein, which is now referred to as ATP7B or Wilson ATPase. Multiple mutations in the gene coding for this ATPase have been found to be associated with Wilson disease, and often more than one mutation contributes to the disease. There is some evidence for an association between genotype and phenotype in Wilson disease, with more severe disease observed in those patients with completely abrogated gene function. However, Wilson disease is one of the most diverse clinical syndromes associated with a single gene defect. The incomplete correlation between specific mutations and clinical manifestations remains unexplained and suggests an important role for modifier genes or unsuspected environmental factors. Knowledge of the Wilson ATPase has yet to be useful in early diagnosis, management, or prevention of this disease, largely because less than half of cases can be identified by screening for the common Wilson ATPase mutations. Instead, diagnosis relies largely on copper and ceruloplasmin testing, as it did before discovery of the Wilson ATPase gene. Copper chelators and zinc supplements continue to be the treatments of choice. The function of the Wilson ATPase and the cellular mechanisms for the regulation of copper pathways involving this transporter remain poorly understood.

Porphyrias: The genes that encode enzymes of the heme synthetic pathways, which are deficient in the hepatic porphyrias, have been identified, cloned, and sequenced, and numerous mutations associated with porphyric phenotypes have been defined. In some situations, DNA analysis can be used for diagnosis and studies of structure-function relationships, as well as genotype-phenotype correlations. However, management of the porphyrias remains difficult. The recent observation of resolution of severe AIP after liver transplantation, demonstrating that the presence of a functional enzyme in the liver alone is sufficient to reverse the disease, has triggered new interest in gene therapy directed at the liver.

The case for an association of PCT with chronic hepatitis C and mild iron overload has been made through the use of newly developed diagnostic tests for hepatitis C and porphyria. However, the mechanisms by which hepatitis C and iron trigger clinical manifestations of PCT remain unclear. Finally, hepatobiliary disease in EPP is caused by the toxic effects of protoporphyrin, which is overproduced mainly in the bone marrow. This finding raises the prospect of bone marrow-based cell or gene therapy for severe cases of EPP.

Cystic Fibrosis: The gene for cystic fibrosis was first identified in 1989 to be an integral membrane chloride transporter (cystic fibrosis transmembrane regulator or CFTR) that is widely expressed on epithelial cells, including cholangiocytes. Multiple mutations in CFTR have been identified; however, at least half of patients with CF are homozygous for one type of mutation, called delta F508. Despite knowledge of the gene that is mutated in CF, the pathogenesis of the lung, pancreatic, and liver injury is not well-understood, and there are no specific therapies that have been shown to prevent or ameliorate the focal biliary cirrhosis that occurs in up to two-thirds of patients with CF.

Congenital Hepatic Fibrosis: Autosomal recessive polycystic kidney disease and congenital hepatic fibrosis occur as a distinct entity resulting from mutations in the PKHD1 gene, which encodes the protein fibrocystin. This large, integral membrane protein, which is expressed on cilia, may interact directly with the EGF receptor in cells of the renal collecting ducts and hepatic cholangiocytes. The precise function of fibrocystin remains unknown, but activation of EGF and its receptor appears to be one of its effects.

In summary, the genetic mutations responsible for several types of genetic liver disease have been identified, but further research is required to translate this knowledge into means of prevention and treatment.

RESEARCH GOALS

The ultimate goals for research on genetic liver diseases are to develop practical and reliable means of screening and diagnosis, as well as to provide a means of control and prevention of their disease manifestations. Research on these genetic diseases will also provide new insights into normal vs. aberrant metabolism of iron, copper, and porphyrins, which are likely to improve diagnosis and treatment of other diseases.

Although the genes of the major forms of genetic liver diseases have been identified, further work is warranted to elucidate the mechanisms of action of the enzymes, transporters, and signaling molecules that constitute the genetic defects in these diseases. It is particularly important to provide structure-function information about the mutated proteins and how they interact with other metabolic pathways. These details are ultimately important for identifying possible targets for intervention to alleviate the disease manifestations and prevent tissue damage.

Hemochromatosis: In order to understand the alterations in iron metabolism that underlie hemochromatosis, it is important to achieve a detailed definition of the normal molecular mechanisms of iron metabolism in humans. A specific research focus of importance is the role of *HFE* and its interactions with hepcidin.

- *Research Goal:* To fully define normal pathways of iron metabolism in humans (Matrix Cell B2).

The pathways of iron metabolism are affected by, and are interwoven with, other cellular pathways and processes such as cytokine signaling, apoptosis, regeneration, and repair. Knowledge of iron metabolism is likely to lead to insights into other diseases and their manifestations, such as alcoholic liver disease, nonalcoholic steatohepatitis (NASH), hepatitis C, and porphyria cutanea tarda (PCT).

- *Research Goal:* To define the role of iron metabolism in NASH, alcoholic liver disease, chronic hepatitis C, and PCT (Matrix Cell B2).

For clinical management of hemochromatosis, it is important to define the frequencies of the *HFE* gene mutations in the general population and how frequently these mutations result in iron overload and disease manifestations.

- *Research Goal:* To more fully define the frequency of disease expression associated with *HFE* mutation (Matrix Cell A1).

However, not all cases of inherited forms of iron overload are due to the classical *HFE* mutations. This is particularly true among African Americans and Asian Americans, who rarely harbor the C282Y mutation yet have severe and unexplained forms of iron overload.

- *Research Goal:* To identify the genetic causes of hemochromatosis in these ethnic groups and to improve screening and molecular diagnosis (Matrix Cell B3).

Because the typical commercial assays for *HFE* mutations will not detect these lesser known causes of hereditary hemochromatosis, expert centers of excellence for DNA diagnostics and sequencing would help to more fully define the genetic causes of iron overload.

- *Research Goal:* To establish centers of excellence for genetic evaluation of hemochromatosis (Matrix Cell A2).

Practical approaches to screening for iron overload and algorithms for molecular diagnosis would arise out of population studies and provide firm medical evidence for recommendations on screening in different populations at different ages.

- *Research Goal:* To develop and apply practical and accurate methods to screen for hemochromatosis before tissue injury occurs (Matrix Cell B1).

Additionally, even after the molecular diagnosis of hemochromatosis is made, patients with identical gene mutations often have markedly different clinical courses and expression of disease, suggesting an important role of gene modifiers in hemochromatosis.

- *Research Goal:* To further define these gene modifiers of hemochromatosis (Matrix Cell A1).

Finally, a major challenge in hemochromatosis is the development of means to diagnose the disease early in persons who are likely to develop severe iron overload and tissue damage. Management of both hereditary and acquired forms of iron overload would be greatly aided by an inexpensive, widely available, noninvasive means to assess total body and hepatic iron content.

- *Research Goal:* To develop a noninvasive means to measure total body and hepatic iron content (Matrix Cell C3).

These noninvasive measures might be imaging tests or mathematical models based upon clinical features and results of blood tests and metabolic assays.

Wilson Disease: While the gene responsible for Wilson disease has been identified and the major mutations associated with disease have been characterized, the pathogenesis of copper overload and resultant cell injury has not been fully defined. Understanding the pathogenesis of this disease would be greatly aided by further knowledge of the processes of copper absorption and metabolism.

- *Research Goal:* To elucidate more completely the molecular mechanisms and pathways of intestinal absorption, hepatic metabolism, and biliary secretion of copper (Matrix Cell A3).

A major focus of research on pathogenesis should be on the role of Wilson ATPase and how mutations cause defects in excretion and disease manifestations (genotype-phenotype correlations). Additionally, heterozygosity for Wilson ATPase may account for unusual manifestations of other liver diseases and could be explored. Also, importantly, the great clinical heterogeneity of Wilson disease that results from the same mutation in the Wilson ATPase remains unexplained.

- *Research Goal:* To develop improved tests for the mutations in Wilson ATPase and increase the availability of resources for testing of clinical materials (Matrix Cell A2).
- *Research Goal:* To define the role of heterozygosity for Wilson ATPase in other liver diseases (Matrix Cell B1).
- *Research Goal:* To define modifier genes in this condition that might explain why patients with the same Wilson ATPase mutations can present with widely different clinical manifestations (e.g., acute liver failure, cirrhosis, psychosis, neurological disease) (Matrix Cell C2).

Therapy of Wilson disease is fairly straightforward, but refinements in management would benefit patients. A major advance in the diagnosis and management of Wilson disease would be the development of a means of assessing hepatic copper concentrations noninvasively, without dependence on liver biopsy.

- *Research Goal:* To develop a noninvasive means of assessing hepatic copper content (Matrix Cell C3).

These resources would facilitate clinical studies of better approaches to management of this disease. Arguably, the most critical goal in Wilson disease research is the development of a practical and reliable means of metabolic diagnosis of Wilson disease that would serve not only as a diagnostic tool, but also as a way to screen the general population for this potentially fatal, but eminently treatable disease.

- *Research Goal:* To develop a metabolic screening test for Wilson disease (Matrix Cell C1).

A reliable screening test that could be applied to newborns or infants would allow for elimination of morbidity and mortality from this disease.

Porphyrias: Although the relevant genes of the hepatic porphyrias have been identified, diagnosis and treatment of these diseases remain problematic. A central issue is the availability of molecular tests for the porphyrias, including sequencing capabilities to define the rarer mutations.

- *Research Goal:* To establish DNA evaluation centers to sequence genetic mutations underlying the porphyrias (Matrix Cell A2).

A clearer definition of the metabolic pathways of heme synthesis and degradation would aid in defining the genotype-phenotype and structure-function relationships in the hepatic porphyrias. The molecular definition of these pathways would also help to define therapeutic targets for these diseases. The development of improved treatments for the acute crises of AIP is an important goal because hematin infusion therapy is expensive and only partially effective.

- *Research Goal:* To develop an improved therapy for acute crises in AIP (Matrix Cell C2).
- *Research Goal:* To further elucidate the pathophysiology of hepatic porphyrias, including the causes of the associated neuromuscular manifestations, the role of alcohol and estrogens in exacerbating porphyria symptoms, and roles of porphyrins in causing an increased susceptibility to HCC (Matrix Cell B3).

Severe forms of porphyria represent excellent candidates for gene therapy. For instance, in EPP, the major source of the toxic protoporphyrin appears to be the bone marrow, and, therefore, bone marrow transplantation or replacement gene therapy may be a means of treatment. Similarly, in AIP, replacement of the defective enzyme in liver may reverse the systemic condition.

- *Research Goal:* To develop practical gene or stem cell therapies for AIP and EPP (Matrix Cell C3; see also Chapters 3, 4 and 10, C3).

Cystic Fibrosis: Although the gene that is mutated in CF has been identified and extensively characterized, the pathogenesis of liver disease in this disorder is not clear. Considerable research is now focused on understanding the function and structure of CFTR and developing an effective gene therapy. An important goal for future research on the liver disease of CF is to elucidate the specific pathogenesis of the liver injury.

- *Research Goal:* To develop an animal model for the liver disease of CF (Matrix Cell A2).

An animal model of CF would allow investigation on the pathogenesis of the inspissation of bile and the steatohepatitis and how these lead to liver injury. Such an animal model would also provide insights into potential targets for prevention or treatment and allow for *in vivo* testing of candidate therapies, as well as means to search for modifying genes.

Congenital Hepatic Fibrosis: Although rare, congenital hepatic fibrosis poses critical challenges that have implications for many common liver diseases. The PKHD1 gene encodes an integral membrane protein (fibrocystin) found in all cholangiocytes; yet, the mechanism of biliary cyst formation and hepatic fibrosis remains unknown. Basic research on the structure and function of this gene and its product, as well as the development of animal models of the liver disease, would help delineate the cause and best treatment of this disease. Clinical research could also help to characterize the disease and to establish the best approach to its management.

- *Research Goal:* To develop a cohort of patients with congenital hepatic fibrosis to define the natural history of this condition and its optimal diagnosis and management (Matrix Cell A1).

STEPS TO ACHIEVE RESEARCH GOALS

A major opportunity to help advance research in each of these genetic liver diseases is the pursuit of molecular diagnosis and studies of genotype-phenotypic comparisons. At present, the only commercially available DNA assays to identify gene mutations in these diseases are available for hemochromatosis to assess the classical C282Y and H63D mutations of *HFE*. However, even in the case of hemochromatosis, several other genes have been associated with rarer forms of this disease, and clearly new genes will be identified in the future associated with inherited forms of iron overload in African American, Hispanic, and Asian American patients. The pursuit of the genetic basis for these other causes of hemochromatosis warrants active support.

Also helpful would be similar genetic studies in Wilson disease and the porphyrias. At present, DNA testing for these diseases is done at a few sites, largely by prominent, NIH-funded basic researchers who take on such screening efforts at the expense of their basic research grants, which do not provide funds for such a resource that lacks a specific hypothesis-driven focus. Further support of state-of-the-art DNA sequencing capabilities, dedicated to characterizing genes associated with these diseases, would provide a nidus for further basic and clinical advances in these diseases. These research project grants could also include a database of patients' clinical information to help with genotype-phenotype correlations and to provide cohorts of well characterized patients for clinical investigation. Increased focus on these genetic diseases could lead to multicenter studies focusing on clinical trials on optimal management of these diseases, such as on the optimal use of zinc supplements and copper-chelating agents in Wilson disease. These centers could also serve as a resource for patients, providing reliable information and fostering communication with other patients and researchers.

Continued research advances on genetic liver diseases will also require collaboration and coordination among NIH Institutes and other biomedical research funding agencies, such as the Department of Veterans Affairs, Centers for Disease Control and Prevention, and Food and Drug Administration, as well as leading private foundations. Specific ad hoc working groups on these diseases might facilitate these interactions and collaborations. The recently initiated clinical research study on the natural history of autosomal recessive polycystic kidney disease and congenital hepatic fibrosis serves as an example of such collaborations. This study is supported by the National Human Genome Research Institute, the Office of Rare Diseases, and the ARPKD/CHF Alliance.

Matrix of Research Goals in Genetic Liver Disease

	Short Term (0-3 years)	**Intermediate Term (4-6 years)**	**Long Term (7-10 years)**
High Risk	**A3.** Fully elucidate the molecular mechanisms of intestinal absorption, hepatic metabolism, and biliary excretion of copper.	**B3.** Identify the major genetic causes of inherited iron overload among African Americans, Asian Americans, and Hispanics. Define the molecular basis of the increase in HCC risk among persons with the porphyrias.	**C3.** Develop noninvasive means of accurately defining total body and hepatic iron and copper, either using imaging studies or mathematical models and serum levels of related molecules. Develop practical gene or stem cell therapy for AIP and EPP.
Intermediate Risk	**A2.** Establish DNA evaluation centers of excellence for Wilson disease, the porphyrias, and hemochromatosis. Develop a reliable animal model for the liver disease of cystic fibrosis.	**B2.** Fully define the normal molecular pathways of iron metabolism in humans with specific definition of the roles of *HFE* and hepcidin. Define the role of liver iron levels in the course of NASH, alcoholic liver disease, chronic hepatitis C, and PCT.	**C2.** Define specific genetic modifiers of Wilson disease and porphyrias using animal models and clinical cohorts of patients. Develop an improved therapy for amelioration of acute crises in porphyria.
Low Risk	**A1.** More fully define the frequency of disease expression associated with *HFE* C282Y and define major modifying factors. Identify a cohort of patients with congenital hepatic fibrosis to study its natural history and optimal management.	**B1.** Develop and apply practical and accurate screening methods for identifying hemochromatosis before significant tissue injury has occurred. Define the role of heterozygosity for Wilson ATPase and *HFE* mutations in other liver diseases.	**C1.** Develop rapid metabolic screening test for Wilson disease that could also be applied to newborns or infants and assess test for efficacy and risk-benefit ratio.